

## MB16

5'- GATCC tctagagtcggc TTTACA ctttatgcttc (cg-gctcg...-3'  
 3'- G agatctcagccg aaatgt gaaatacgaag gc (cgagc...-5'  
 5       |                                   | -35 |                                   |  
       BamHI   HpaII

## MB22 insert

10       5'- GATCC actcccatcccccctg TTGACA attaatcat -3'  
       3'- G tgaggggtagggggac AACTGT taattagtagc-5'  
           |                                   | -35 |                                   |  
           BamHI   (HpaII)  
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Promoter and RBS variants of the fusion protein gene were constructed by basic DNA manipulation techniques to generate the following:

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	Promoter	RBS	Encoded Protein.
pGEM-MB16	lac	old	VIIIIs.p.-BPTI-matureVIII
pGEM-MB20	lac	new	" "
pGEM-MB22	tac	old	" "
25   pGEM-MB26	tac	new	" "

The synthetic gene from variants pGEM-MB20 and pGEM-MB26 were recloned into the altered phage vector M13-MB1/2 to generate the phage constructs designated  
 30   M13-MB27 and M13-MB28 respectively.

## iii. Signal Peptide Sequence.

In vitro expression of the synthetic gene regulated by tac and the "new" RBS produced a novel protein  
 35   of the expected size for the unprocessed protein (about 16 kd). In vivo expression also produced novel protein of full size; no processed protein could be seen on phage or in cell extracts by silver staining or by  
 40   Western analysis with anti-BPTI antibody.